

Effects of selective COX-2 inhibition on allergen-induced bronchoconstriction and airway inflammation in asthma

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Background: Prostaglandins that constrict and relax airways are synthesized in reactions catalyzed by either COX-1 or COX-2. It is not known whether selective inhibition of COX-2 makes asthmatic responses better or worse.

Objective: To determine the effects of the selective COX-2 inhibitor, etoricoxib, on allergen-induced bronchoconstriction in asthmatic subjects.

Methods: Sixteen subjects with mild atopic asthma underwent rising dose inhalation challenges with allergen or methacholine to determine PD₂₀ FEV₁ during a control study period or after 10 to 13 days of treatment with etoricoxib (90 mg once daily). The order of study periods was randomized with at least 2-week washout periods. Induced sputum cells and fractional exhaled nitric oxide levels were used to assess airway inflammation and blood assays for COX-1 and COX-2 activity to assess enzyme inhibition. Urinary excretion of lipids was used to assess prostaglandin biosynthesis.

Results: Etoricoxib did not change baseline lung function, nor airway responsiveness to allergen or to methacholine. Neither were the allergen-induced increase in sputum eosinophils and fractional exhaled nitric oxide levels affected by treatment. The biochemical effectiveness of the treatment was established both in the blood assays and by the distinct inhibitory effect of etoricoxib on urinary excretion of tetranor-prostaglandin E₂ ($P < .001$).

Conclusions: This first study of COX-2 inhibition in provoked asthma found no negative effects of etoricoxib on allergen-induced airflow obstruction and sputum eosinophils, basal lung function, or methacholine responsiveness. The study suggests

that short-term use of COX-2 inhibitors is safe in subjects with asthma. (*J Allergy Clin Immunol* 2014;134:306-13.)

Key words: Asthma, COX, NSAIDs, eicosanoids, prostaglandin, thromboxane, leukotrienes, airway hyperresponsiveness, mass spectrometry, urinary metabolites

Prostaglandins are lipid mediators of inflammation and capable of both constricting and relaxing the airways.¹ Prostaglandins are synthesized by either of 2 COX isoenzymes, the constitutively expressed COX-1, active under basal conditions, or COX-2, which is often induced during inflammation.¹ Although traditional nonsteroidal anti-inflammatory drugs (NSAIDs) block both COX isoforms, the coxibs preferentially inhibit COX-2.

In the current study, we hypothesized that the inhibition of COX-2 might be associated with an exaggerated airway response to allergen challenge, by decreasing formation of the bronchodilator prostaglandin E₂ (PGE₂)² while maintaining production of the bronchoconstrictor prostaglandin D₂ (PGD₂).³ This hypothesis was based on the results of a previous study in which we found that basal biosynthesis of PGD₂ was increased in asthmatic subjects and its formation was catalyzed exclusively by COX-1.⁴ In contrast, the same study found that COX-2 contributed substantially (>65%) to whole-body PGE₂ biosynthesis. Although PGE₂ is predominantly considered to be proinflammatory in most parts of the body, it appears to have mainly protective and anti-inflammatory effects in the airways. In mouse models of asthma, it has been suggested that the inhibition of COX-2 leads to enhanced airway hyperresponsiveness.^{5,6} In subjects with asthma, inhalation of prostaglandin E (PGE) inhibits the release of cysteinyl-leukotrienes and attenuates the bronchoconstriction induced by allergen and other indirect stimuli.^{2,7-9} Our primary aim was therefore to examine whether COX-2 inhibition triggers a proasthmatic imbalance in the prostaglandin system, by blocking the protective PGE₂ while maintaining the biosynthesis of disease-driving PGD₂.

The clinical relevance of this study was therefore to provide information concerning the safety of COX-2 inhibitors in patients with asthma. Although coxibs are unsuitable for chronic treatment of inflammation due to cardiovascular side effects,¹ they are helpful in the short-term alleviation of acute episodes of pain or inflammation due to minimal effects on bleeding and gastrointestinal integrity. It was therefore considered important to assess the effects of short-term use of COX-2 inhibitors in asthmatic subjects in a setting in which experimental worsening of asthma was induced, especially as asthmatic subjects in general are often advised to avoid NSAIDs because of the potentially life-threatening reactions that may occur in patients with a well-defined subphenotype of asthma, aspirin-exacerbated

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Abbreviations used

FENO:	Fractional exhaled nitric oxide
NSAIDs:	Nonsteroidal anti-inflammatory drugs
PGD ₂ :	Prostaglandin D ₂
PGE:	Prostaglandin E
PGE ₂ :	Prostaglandin E ₂
PGF _{1α} :	Prostaglandin F _{1α}
PGI ₂ :	Prostaglandin I ₂
TXA ₂ :	Thromboxane A ₂
TXB ₂ :	Thromboxane B ₂

respiratory disease. In these patients, exacerbations are precipitated by the intake of aspirin and other NSAIDs that inhibit COX enzymes.¹⁰ In view of the many indications for NSAIDs in the treatment of inflammation, fever, and pain, this recommendation is both unfortunate and probably often unnecessary. While there is good evidence that coxibs are tolerated by patients with aspirin-exacerbated respiratory disease, at least when they are clinically stable,¹¹⁻¹³ the safety of COX-2 inhibitors in subjects with asthma in general has not been established.

In this study, the effect of COX-2 inhibition on induced asthmatic airway obstruction and inflammation was therefore investigated by intervention with one of the most specific COX-2 inhibitors, etoricoxib.¹⁴ Bronchial allergen challenges were performed to produce a graded and standardized airway obstruction. This is a safe way to mimic a naturally occurring exacerbation with a well-established clinical relevance.¹⁵ Fractional exhaled nitric oxide (FENO) and eosinophils in induced sputum were analyzed as biomarkers of altered asthmatic airway inflammation. To confirm the effectiveness and selectivity of COX inhibition, standard functional blood assays were used.¹⁶ Finally, the effect of the allergen challenge and COX-2 inhibition on the biosynthesis of prostanoids was determined by using mass spectrometric analysis of urinary prostaglandin metabolites.¹⁷

METHODS

Subjects

Sixteen nonsmoking subjects, aged 18 to 55 years, with mild atopic asthma, an FEV₁ of 75% or more of predicted normal, and a positive methacholine bronchoprovocation were recruited from our clinic and via advertisement. The subjects were treated only with short-acting bronchodilators and had no respiratory disease other than asthma, and no respiratory infection in the 4 weeks before inclusion. Exclusion criteria included hypersensitivity to coxibs, and a history of cardiovascular disease, including hypertension. The use of paracetamol, NSAIDs, and other anti-inflammatory drugs was not allowed during the study.

The Ethical Review Board in Stockholm approved the study (Dnr 2009/959-31-4, 2009/1422-32), and the subjects gave written informed consent.

Study design

The study comprised a screening phase followed by a randomized 2-period, cross-over comparison between active treatment with the selective COX-2 inhibitor, etoricoxib, and an untreated control period with identical design (Fig 1). Those carrying out analysis of sputum and biochemical end points were unaware of which period the active treatment was given.

At screening, data on baseline characteristics including FENO, FEV₁, skin prick testing, specific IgE for the allergen selected to be used in the challenges, and current airway sensitivity to methacholine and allergen were obtained (Table I). A washout of at least 14 days preceded the start of the cross-over phase, which comprised 3 clinic visits during each period (Fig 1). Etoricoxib (Arcoxia; Merck Sharp & Dohme, South Granville, Australia) tablets 90 mg

were purchased from the hospital pharmacy and administered once daily for 10 to 13 days, with the first dose taken in the clinic after baseline assessments on study day 1 of the treatment period. A methacholine challenge was performed on the first and the penultimate day of each period, and an allergen inhalation challenge was then performed on the last day of each period. Sampling of blood was performed at baseline (study day 1), and on the last 2 days of each period (study days 2 and 3, 1 hour before methacholine and allergen challenges, respectively). Sputum induction was performed 1 hour after methacholine challenge on study days 1 and 2 and at 6 hours after the maximum fall in FEV₁ following allergen provocation on study day 3 (Fig 1). Urine was collected before the start of allergen bronchoprovocation, and at 1 and 2 hours after the maximum fall in FEV₁.

Bronchoprovocations

Inhalation challenges were performed as previously described¹⁸ by using a dosimeter-controlled jet nebulizer (Spira Electro 2; Respiratory Care Center, Hameenlinna, Finland) and with pulmonary function measured as FEV₁. Allergen and its diluent (Aquagen) were purchased from ALK Laboratories (Copenhagen, Denmark) and methacholine from Norrland's University Hospital Pharmacy (Umea, Sweden). Challenges were always performed in the morning and started by inhalation of the diluent. Provided the FEV₁ did not change by more than 10%, inhalation of methacholine or the allergen to which the subjects were sensitized was commenced with the postdiluent FEV₁ value used as baseline. Half-log increments in the cumulated dose of allergen were inhaled every 15 minutes (7-7100 SQ units), whereas methacholine was administered every third minute in doubling doses (14.2-7256 μg). The challenge was stopped when the FEV₁ had fallen by at least 20%. The PD₂₀ value was derived by linear interpolation from the log cumulated dose-response curve.

Measurement of FENO

FENO (NIOX analyzer; Aerocrine AB, Solna, Sweden) was measured at a flow rate of 50 mL/s according to American Thoracic Society guidelines.¹⁹

Sputum induction

Briefly, subjects inhaled 0.2 mg albuterol and provided the FEV₁ was 70% or more of predicted inhaled an aerosol (DeVilBiss Ultraneb 3000; Dolema AB, Täby, Sweden) containing increasing concentrations of saline (3%, 4%, and 5%) for 7 minutes each.²⁰ Spirometry was obtained after each concentration, and the induction was stopped only if the FEV₁ declined by 20%. Sputum plugs were extracted from the sample and processed within 2 hours as described.²⁰ Cell viability was assessed by using trypan blue solution (0.4%), and cells were classified as viable, nonviable, and squamous; the accepted proportion for the latter was less than 20%. Cytospins were stained with May-Grünwald-Giemsa solution, and total and differential nonsquamous cell counts were performed.

COX-1 and COX-2 assays

COX-1 activity was assessed by thromboxane B₂ (TXB₂) generation in clotted blood (1 hour, 37°C), and COX-2 activity was assessed by the formation of PGE₂ in heparinized blood stimulated with LPS (100 μg/mL; 24 hours, 37°C), as previously described,¹⁶ using enzyme-immunoassays (Cayman Chemical, Ann Arbor, Mich). The biological activity of etoricoxib was validated by the addition of the drug *ex vivo* (0.1-10 μM) to blood drawn from the subjects during the control arm of the study. Results confirmed a dose-dependent inhibition of LPS-induced PGE₂ formation with an IC₅₀ of 0.78 μM (Table II). Conversely, it was documented that the addition of etoricoxib *ex vivo* had no effect on TXB₂ levels (Table II).

Collection of urine and measurement of urinary metabolites

Urine was stored at -70°C until assayed. Metabolites of prostaglandins, thromboxane, and leukotriene E₄ were measured by the use of ultra

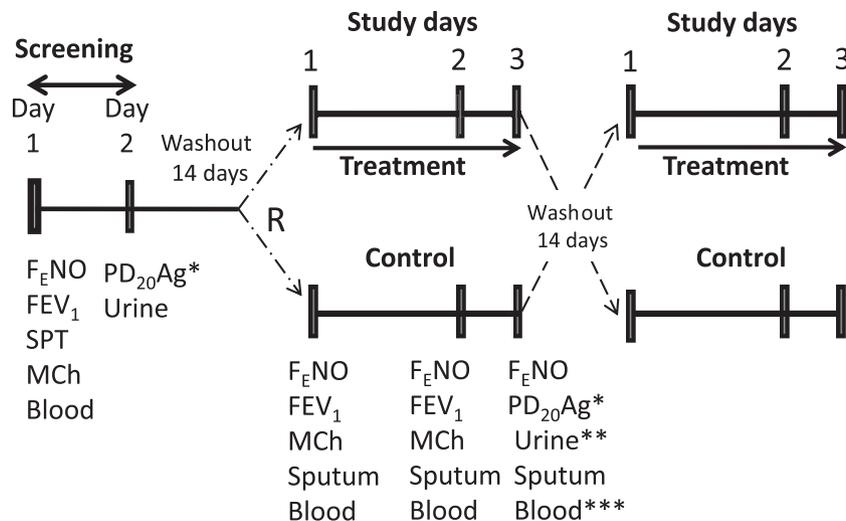


FIG 1. Study design: Two randomized periods with etoricoxib 90 mg once daily for 10 to 13 days vs control period with no treatment. *MCh*, Methacholine challenge; *R*, randomization; *study day 1*, baseline data before start of period; *study day 2*, effects of etoricoxib/no treatment baseline data; *study day 3*, effects of etoricoxib/no treatment on allergen-induced airway obstruction, urinary mediators, and inflammatory cells in induced sputum. *Cumulative allergen challenge. **Urine before and 1 and 2 hours after allergen challenge. ***Blood before allergen challenge.

TABLE I. Patients' characteristics

Patient	Age (y)	M/F	FENO (ppb)	FEV ₁ (L)	FEV ₁ % predicted	Allergen	Specific IgE level (kU/L)	Methacholine PD ₂₀ (μg)	Allergen PD ₂₀ (SQ unit)
1	24	M	20	5.72	109	Cat	17	122	276
2	49	M	11	3.43	105	Cat	14	359	122
3	35	M	23	3.82	91	Birch	4.5	30.3	37.7
4	32	F	25	3.18	93	Cat	66	226	81.6
5	23	M	15	3.96	94	Birch	19	549	825
6	26	M	49	5.21	110	Cat	27	57.3	130
7	26	M	18	4.18	98	Birch	46	565	334
8	44	M	18	5.01	118	Grass	14	469	16.3
9	44	M	37	3.6	95	Cat	6.5	4878	2040
10	33	F	15	3.34	104	Birch	14	2764	1015
11	50	M	39	3.8	98	Grass	3.1	172	346
12	27	F	14	3.4	98	Cat	43	343	17.3
13	35	F	19	3.66	112	Cat	22	227	69.9
14	28	F	100	3.35	108	Cat	62	39.6	244
15	45	M	9	3.86	107	Cat	1.6	87.8	240
16	31	M	18	4.36	104	Grass	0.76	106	4804
Mean	34.5		26.9	3.99	102.75		22.53	244.8*	210.378*
Range	23-50		9-100	3.18-5.72	91-118		1.6-66	30.3-4878	16.3-4804

F, Female; FEV₁, forced expiratory volume in 1 second; FENO, fraction of exhaled nitric oxide; M, male; PD₂₀, dose provoking a fall in FEV₁ of 20% or more.

*Geometric mean.

performance liquid chromatography/tandem mass spectrometry and expressed as nanogram per hour.¹⁷

blood pressure, urinary metabolites, blood COX-assays, and FENO (log-transformed values).

Statistical analysis

PD₂₀ FEV₁ values for methacholine and allergen were logarithmically transformed, paired *t* tests were performed, and data were presented as geometric mean and range. Analysis by repeated-measures ANOVA within and between the study arms was performed to determine the changes in the percentage of differential cell counts, and results were presented as mean and SEM. Absolute sputum cell numbers were not normally distributed and were compared by using the Wilcoxon signed rank test. Paired *t* tests were used to determine changes in lung function,

RESULTS

Effects of etoricoxib on lung function, systemic arterial blood pressure, and safety variables

All 16 included subjects completed the study protocol and were treated with etoricoxib for 10 to 13 days before allergen challenge. Neither prechallenge baseline lung function nor systolic or diastolic blood pressure exhibited any differences between the 2 periods (Table III). The active

TABLE II. Effect of etoricoxib added *in vitro* on COX-1 and COX-2 activity

	Control	0.1 μ M Etoricoxib	1 μ M Etoricoxib	10 μ M Etoricoxib
PGE ₂ (ng/mL)	31.0 (7.6)	28.6 (6.8)	13.3 (3.4)*	1.8 (0.3)*
TXB ₂ (ng/mL)	188.6 (25.7)	196.8 (31.9)	196.7 (31.1)	166.7 (29.2)

Results shown as mean (SEM). Blood was collected during the untreated arm of the study. COX-1 activity was assessed by TXB₂ generation in clotted blood (1 h, 37°C), and COX-2 activity was assessed by PGE₂ levels in blood incubated with LPS (100 μ g/mL, 24 h, 37°C).

* $P < .05$ compared with control (no etoricoxib).

TABLE III. Blood pressure (BP) and baseline lung function (FEV₁) control period versus treated period

	Control period		Treated period	
	Start	End	Start	End
FEV ₁ (L), mean \pm SD	3.9 \pm 0.7	3.8 \pm 0.7	3.8 \pm 0.7	3.8 \pm 0.7
FEV ₁ (% predicted), mean \pm SD	100.5 \pm 8.7	99.7 \pm 9.8	97.9 \pm 11.6	99.1 \pm 12.0
BP systolic, mean \pm SD	114 \pm 9.1	113 \pm 8.1	115 \pm 7.3	115 \pm 9.3
BP diastolic, mean \pm SD	75 \pm 5.6	74 \pm 5.0	74 \pm 5.2	73 \pm 7.2

treatment was well tolerated with no significant adverse events.

Effects of etoricoxib on response to allergen bronchoprovocation

Etoricoxib did not affect the airway sensitivity to inhalation of cumulatively increasing doses of allergen. The geometric mean PD₂₀ FEV₁ was 234 (range, 31.7-5244) and 200 (range, 12.2-3198) standardized quality units after drug and control, respectively (Fig 2, A). Etoricoxib was also without effect on the immediate peak fall in FEV₁ (30.4% \pm 1.4% after etoricoxib vs 28.1% \pm 1.7% during control session; $P > .5$) and the decrease in FEV₁ observed after 30 minutes (19.4% \pm 1.8% after etoricoxib vs 17.7% \pm 1.9% during control session; $P > .5$). Furthermore, in the subgroup of 9 asthmatic subjects who inhaled the same allergen dose on both occasions, there was no difference in the magnitude of the maximum fall in FEV₁ (29% \pm 2.0% and 26% \pm 1.9% after drug and control, respectively), or in the fall of FEV₁ at 30 minutes after the challenge (17% \pm 2.4% and 15% \pm 2.5% after drug and control, respectively).

Effects of etoricoxib on response to methacholine

Airway responsiveness to methacholine was assessed at the end of the treatment period on the day before the allergen provocation (Fig 1). Methacholine responsiveness, expressed as PD₂₀ FEV₁, was not affected by etoricoxib, the geometric mean being 229 (range, 29-4655) and 222 (range, 56-2018) μ g after drug and control respectively (Fig 2, B).

Effects of etoricoxib on sputum cells and FENO

The total number of cells and the percentage of eosinophils in induced sputum significantly increased 6 hours after

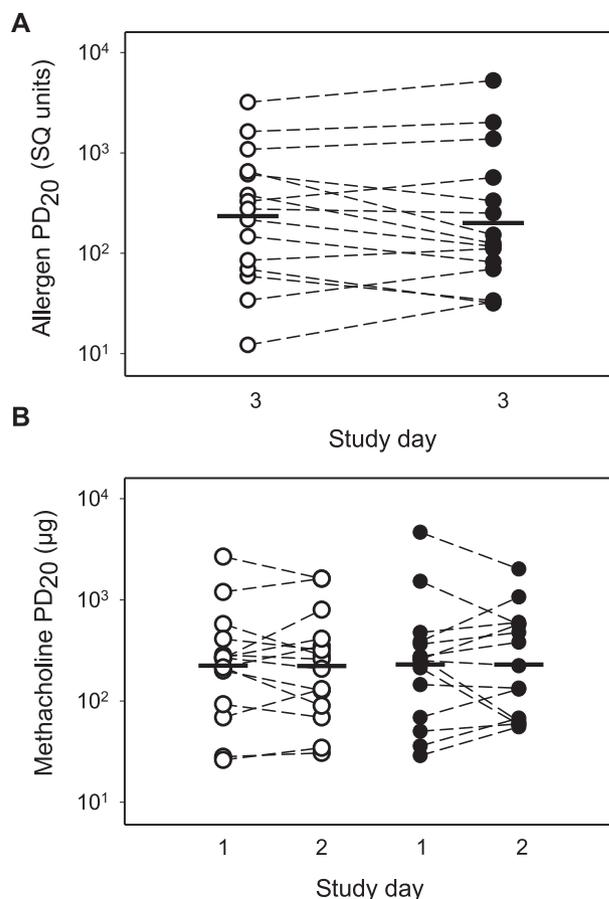


FIG 2. Effects of etoricoxib on airway sensitivity to allergen (A) and methacholine (B), expressed as individual PD₂₀ values. Filled circles represent the etoricoxib treatment period, and open circles represent the control period. Horizontal lines indicate geometric mean values. Study days 1 and 2 represent the first day (baseline) and the penultimate day of each period, respectively.

allergen challenge during both sessions (Fig 3, A and B). Likewise, the increase in the number of eosinophils after allergen challenge was not different during the etoricoxib arm and the control arm (eosinophil numbers [$\times 10^3$ /mg sputum] on day 3 during the control arm were 0.73 [0.39-1.60] and on day 3 during the etoricoxib arm were 1.16 [0.25-2.36], median [interquartile range]). Baseline sputum cell numbers and differential cell counts in samples collected during the etoricoxib and the control periods were also similar (Fig 3, A and B). Furthermore, treatment with etoricoxib did not give rise to any significant changes in FENO within or between the study periods (Fig 4).

Effects of etoricoxib on blood COX-1 and COX-2 activity

COX-2 activity, measured as PGE₂ formation in whole blood incubated for 24 hours with LPS, was consistently inhibited after *in vivo* treatment with etoricoxib but not during the untreated session (Fig 5, A). COX-1 activity was assessed as the level of serum TXB₂ after 1 hour of *ex vivo* clotting. There were no differences in TXB₂ levels between samples collected after etoricoxib or after the control session (Fig 5, B).

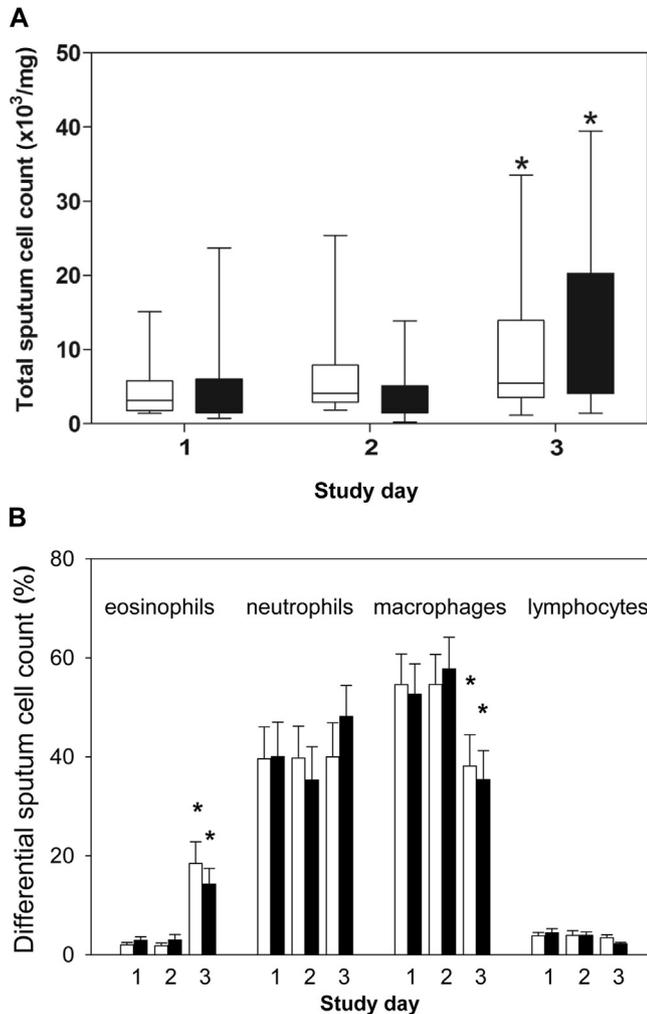


FIG 3. Effects of etoricoxib on the total cell counts (*box and whisker plots* showing median, 25th and 75th percentiles, and min/max) (**A**) and differential cell counts in induced sputum expressed as percentages of total (mean [SEM]) (**B**). *Black bars* represent the treated period, and *open bars* represent the control period. Study days 1, 2, and 3 represent the first (baseline), penultimate, and last day (after allergen provocation) of each period, respectively. * $P < .05$ compared with study day 1.

Effects of etoricoxib on urinary prostaglandin metabolites and leukotriene E₄

During the control arm of the study, there was increased excretion of tetranor-PGD₂ and 2,3-dinor TXB₂ in urine during the hour following the peak drop in FEV₁, indicating increased biosynthesis of PGD₂ and thromboxane A₂ (TXA₂) (Fig 6, A and B). The PGD₂ metabolite increased the most and was still higher than baseline at 2 hours after peak drop, whereas excretion of 2,3-dinor TXB₂ had returned to baseline during the second hour after the challenge.

In contrast, there was no change after the challenge in the urinary excretion of the PGE₂ and prostaglandin I₂ (PGI₂) metabolites tetranor-PGE₂ and 2,3-dinor-6-keto prostaglandin F_{1α} (PGF_{1α}), respectively (Fig 6, C and D). The baseline excretion of tetranor-PGE metabolite was however significantly higher in men than in women (273 ± 80 vs 49 ± 18 ng/h; $P < .01$ Mann-Whitney test), whereas there were no gender differences with respect to the other metabolites.

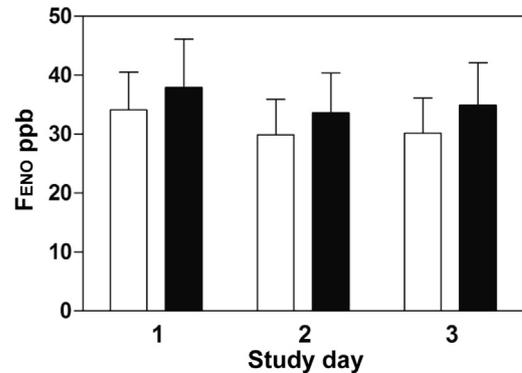


FIG 4. Effect of etoricoxib on FENO measurements (geometric mean [SEM]). *Black bars* represent etoricoxib treatment, and *open bars* represent the control period. Study days 1, 2, and 3 represent the first day (baseline), penultimate day, and last day (before allergen provocation) of each period, respectively.

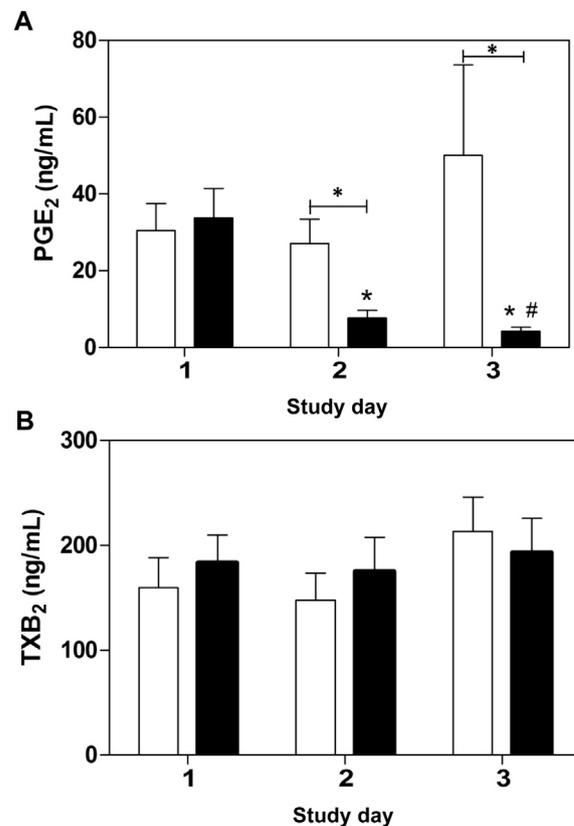


FIG 5. Assessment of COX-2 activity (**A**) and COX-1 activity (**B**) in plasma and serum, respectively. COX-1 activity was assessed by TXB₂ generation in clotted blood (1 hour, 37°C), and COX-2 activity was assessed by PGE₂ levels in blood incubated with LPS (100 μg/mL; 24 hours, 37°C). *Black bars* represent etoricoxib treatment, and *open bars* represent the control period (mean [SEM]). Study days 1, 2, and 3 represent the first day (baseline), the penultimate day, and the last day (before allergen provocation) of treatment, respectively. Significant comparisons between etoricoxib and control arms are indicated, as well as comparison with study day 1 (* $P \leq .001$) and comparison with study day 2 (# $P < .05$).

Treatment with etoricoxib reduced basal levels of the urinary PGE₂ metabolite by more than 75%, and the levels remained depressed to this level in samples collected after the challenge

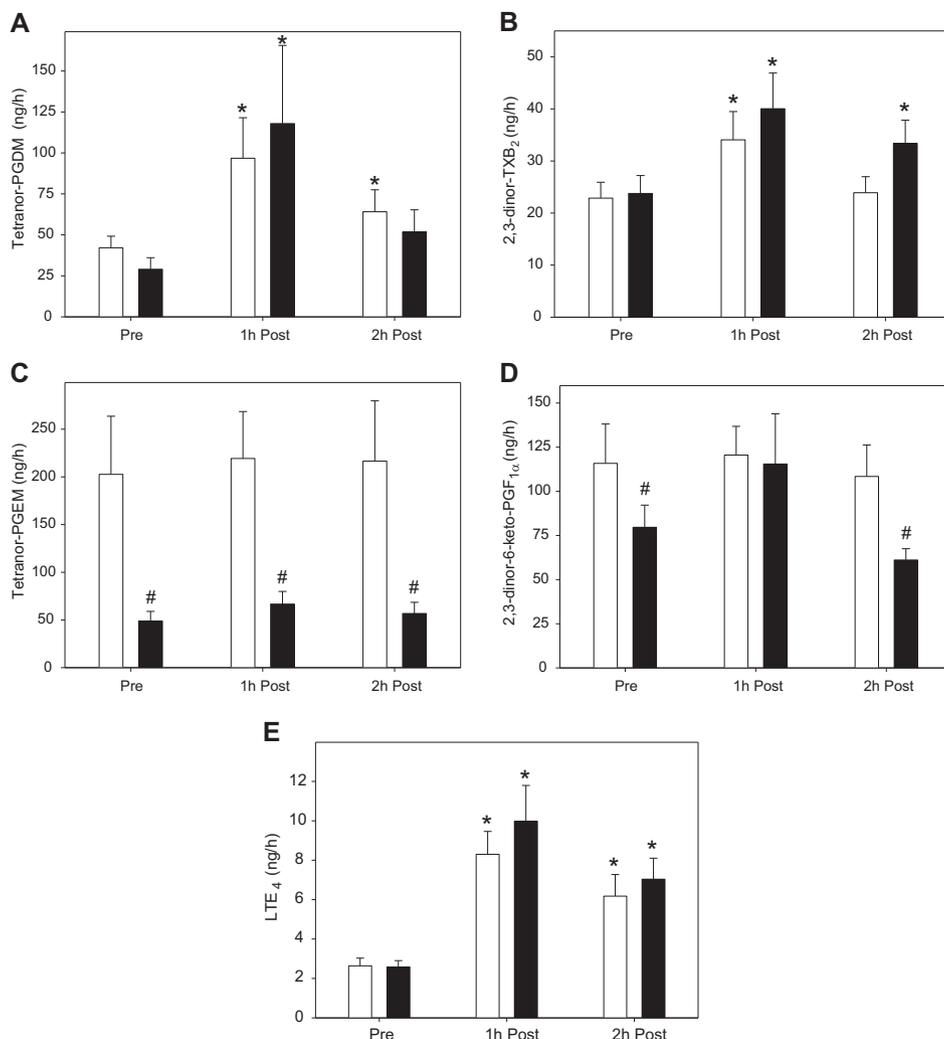


FIG 6. Effects of etoricoxib on metabolites in urine. **A**, Tetranor-PGDM. **B**, 2,3-DinorTXB₂. **C**, Tetranor-PGEM. **D**, 2,3-Dinor-6-ketoPGF_{1α}. **E**, LTE₄. LTE₄, Leukotriene; PGDM, prostaglandin D metabolite; PGEM, PGE metabolite. Patients were untreated (open bars) or treated with etoricoxib (black bars). Urine was collected before provocation (Pre) and 1 and 2 hours after allergen provocation (1 hour post and 2 hour post, respectively). Data are normalized per hour. Error bars represent SEM. *Indicates significantly different from value before provocation ($P < .05$). #Indicates significantly different ($P < .05$) from untreated.

(Fig 6, C). Etoricoxib also caused about a 30% decrease in the basal excretions of the PGI₂ metabolite in urine (Fig 6, D), whereas neither the basal nor the allergen-induced levels of urinary TXA₂/PGD₂ metabolites were affected by treatment with etoricoxib (Fig 6, A and B). There was a consistent and significant increase in urinary leukotriene E₄ level after the allergen challenge, with no difference between treatment and control arms (Fig 6, E).

DISCUSSION

This is the first investigation of the effect of COX-2 inhibition on airway homeostasis in patients with atopic asthma after allergen challenge. The study documents that 10 to 13 days of treatment with etoricoxib had no effect on baseline lung function, airway responsiveness to methacholine, sensitivity to allergen, or the magnitude of the fall in FEV₁ following the PD₂₀ allergen

dose. Neither was there a difference in the allergen-induced increase in sputum eosinophils between the 2 study sessions, or in the surrogate marker of airway inflammation, FENO. The study therefore supports the notion that short-term use of a coxib is safe in asthmatic subjects, even during a mild asthma attack. This confirms and extends our previous observations that showed that baseline lung function in subjects with asthma was unaffected by another COX-2 inhibitor, celecoxib.^{4,13}

Our conclusions can be directly attributed to the inhibition of COX-2, as the biochemical effects of the intervention were proven by 2 different strategies. First, by using validated blood assays for COX-1 and COX-2 inhibition,¹⁶ we confirmed that the study participants had complied with the treatment and that only COX-2 activity, measured as LPS-induced PGE₂ formation, was inhibited. In contrast, there was no effect on COX-1 activity as monitored by TXA₂ formation in clotted whole blood. Second, by using a recently developed mass

spectrometry platform for measuring prostaglandin metabolites,¹⁷ we also replicated our previous observations with celecoxib,⁴ namely, that there was a major (>75%) inhibition in urinary excretion of the main PGE₂ metabolite, PGEM, whereas urinary excretion of the corresponding major tetranor metabolite of PGD₂, PGMD, was unaffected.

Effective COX-2 inhibition can therefore be achieved in subjects with asthma without risk of increased airway responsiveness, either to the directly acting bronchoconstrictor methacholine or to the indirect effects of inhaled allergen acting on mast cells to trigger mediator release. These findings not only provide clinically useful information but also increase our understanding of the pathways involved in the biosynthesis of prostaglandins in the airways. As such, neither basal nor provocation-induced production of the bronchoconstrictive and proinflammatory mast cell product PGD₂ was affected by treatment with etoricoxib, demonstrating that COX-1 is the only enzyme of importance for PGD₂ formation relevant to lung function in humans. It should be recognized that subjects in this study did display signs of ongoing airway inflammation including increased FENO, elevated baseline sputum eosinophils (>2%), and airway hyperresponsiveness to methacholine. This suggests that under conditions of airway inflammation, there is minimal COX-2 involvement in PGD₂ biosynthesis. This is consistent with the results of a biopsy study, showing that in asthmatic subjects during the pollen season there was no increase in the expression of COX-2 or prostaglandin D synthase but a distinct increase in enzymes of the leukotriene pathway.²¹

Furthermore, our data suggest that the pool of PGE₂, which controls airway dynamics, is not affected by systemic COX-2 inhibition, despite the pronounced involvement of COX-2 in whole-body PGE₂ formation. This interpretation is consistent with *in vitro* observations demonstrating that COX-1 is responsible for PGE₂ biosynthesis in human airway epithelium.²² The results of this trial are also in line with the replicated documentations of bronchoconstriction in aspirin-exacerbated respiratory disease being triggered by nonselective NSAIDs, and not by COX-2 inhibitors.¹¹⁻¹³ The absence of increased excretion of the PGE₂ metabolite after the allergen challenge supports the concept that PGE₂ mainly serves to regulate airway tone,²³ and the increased levels in males confirm previous data (eg, Daham et al⁴).

Moreover, the current study provides an increased understanding of the role of the 2 COX isoenzymes in the biosynthesis of prostaglandins in allergen-induced airway obstruction. Although some early studies suggested that traditional NSAIDs affected different components of the response to allergen challenge in subjects with asthma (eg, Fairfax et al²⁴ and Fish et al²⁵), the studies generally suffered from several shortcomings (short duration of treatment, no documentation of biochemical efficacy, short washout periods, etc). More recent studies have shown that systemic treatment with NSAIDs has no effect on either the early or late reaction to allergen challenge.^{26,27} Together with the present data on selective COX-2 inhibition, it is now possible to conclude that COX-1-catalyzed reactions maintain both constrictive (presumably mainly TXA₂ and PGD₂) and relaxant (presumably mainly PGE₂) COX products in the airways. Future studies with selective receptor antagonists or selective inhibitors of distal class-specific isomerases (eg, prostaglandin D synthases or PGE synthases) are required to define the role of individual

prostaglandins in allergen-evoked reactions. For example, PGE₂ exerts its actions at 4 different EP receptors, and may even cause opposing effects at similar concentrations.²³ Previous studies with TP receptor antagonists, which block the bronchoconstrictive effects of PGD₂, have also identified a component of the allergen response that is due to the actions of PGD₂ and TXA₂.^{28,29} More recent data have demonstrated that PGD₂, which is increased in severe asthma,³⁰ also acts on DP₂/CRTH2 receptors,³¹ and it will probably be necessary to block several of its receptors to conclusively establish the role of PGD₂.

The allergen provocation was also associated with significantly increased urinary excretion of 2,3-dinorTXB₂, indicating increased biosynthesis of TXA₂. The latter finding confirms previous observations of TXA₂ release during allergen challenge,^{32,33} as well as during acute attacks of asthma.³⁴ TXA₂ is a potent bronchoconstrictor,³⁵ and its effects on TP receptors might explain the protective effects of TP antagonists observed in allergen-induced bronchoconstriction.^{28,29} The platelet is implicated as the source of TXA₂ in this setting, because low-dose aspirin blocked the allergen-induced increase in urinary excretion of 2,3-dinorTXB₂.³² Platelets may also contribute to COX-1-dependent formation of PGD₂,³⁶ but it is not known whether that pathway is activated by inhaled allergen.

The finding that 2,3-dinor-6-ketoPGF_{1α} did not increase after allergen challenge is also consistent with previous data.³² The substantial inhibition of baseline urinary excretion of 2,3-dinor-6-ketoPGF_{1α} is consistent with the evidence from mice and humans that COX-2 is the major contributor to the biosynthesis of PGI₂.^{37,38}

For this investigator-initiated trial, it was not possible to obtain placebo for etoricoxib from the manufacturer. We consider this as a minor limitation because the study end points involved objective outcomes with minimal sensitivity to subjective bias. It has, for example, been shown that quality of life can be manipulated by persuasion, whereas lung function cannot,³⁹ and, as stated in Methods, involved staff members were unaware of the study period.

Our current findings in subjects with asthma lend a critical perspective to the predictive value of mouse models for exploring the role of COX metabolites in asthmatic responses. Targeting the COX-2 pathway has been reported to have distinct effects, both on airway hyperresponsiveness and on airway inflammation, in standard murine models of allergen challenge,^{5,6} but the effects were obviously not the same in allergen-challenged asthmatic subjects. One reason for this discrepancy relates to species differences regarding the function of eicosanoids in airways. Although PGD₂ is a potent bronchoconstrictor in humans,³ it evokes bronchodilation in mice.⁴⁰

In conclusion, selective COX-2 inhibition does not appear to exert any negative effects on functional and inflammatory responses during the immediate phase in subjects with mild atopic asthma. Mechanistically, the data suggest that the biosynthesis of both the bronchoconstrictive PGD₂ and the bronchoprotective PGE₂ occur via the COX-1 pathway in the airways, at least in subjects with mild asthma with stable disease. Taken together, this provides evidence that short-term use of COX-2 inhibitors may be acceptable in this group of patients. It remains possible that there is an upregulation of COX-2 in severe asthma, and the current findings therefore warrant an assessment of the safety of coxibs in severe asthma.

Clinical implications: Etoricoxib does not worsen allergen-induced airway inflammation or airway obstruction in mild allergic asthma. Hence, short-term use of COX-2 inhibitors may be safe in this asthma phenotype.

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